

Multi-year and multi-environment performance and yield stability of advanced bread wheat lines in the Black Sea Region of Türkiye

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Research Article

Keywords: AMMI, bread wheat, GGE biplot, multi-environment trial, multi-year evaluation, WAAS, yield stability

Posted Date: October 21st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11245661/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Genotype-by-environment interaction is a major challenge in selecting high-yielding and stable bread wheat genotypes. This study evaluated 20 advanced bread wheat lines and five check cultivars over three consecutive growing seasons (2022–2023, 2023–2024, and 2024–2025) in the Black Sea Region of Türkiye.

Results

The dataset comprised seven location × year environments: Bafra and Tokat in 2022–2023 and 2023–2024, and Bafra, Çarşamba, and Tokat in 2024–2025. The 2022–2023 records came from unreplicated preliminary yield trials, whereas later seasons used replicated trials. Because precision differed among seasons, the principal multi-year stability analysis used genotype × environment grain-yield means; the replicated 2024–2025 dataset was additionally analyzed at plot level for grain yield, hectoliter weight, and thousand-kernel weight. Across seven site-years, mean grain yield was 6063.5 kg ha⁻¹, with environment means ranging from 2883.7 kg ha⁻¹ in Bafra 2022–2023 to 8795.3 kg ha⁻¹ in Tokat 2023–2024. Line-4 had the highest overall mean yield (6794.3 kg ha⁻¹), followed by Line-9 (6630.3 kg ha⁻¹) and Kirve (6545.6 kg ha⁻¹). Excluding the two unreplicated preliminary environments produced significant concordance between seven-site-year and five-replicated-site-year genotype rankings (Spearman $\rho = 0.705$, $p < 0.001$), with Line-4 remaining first. AMMI IPCA1 and IPCA2 explained 46.68% and 20.92% of the $G \times E$ interaction, while GGE PC1 and PC2 explained 39.82% and 22.65% of $G + G \times E$ variation. Line-4 also had the lowest WAAS (110.17).

Conclusions

The seven-environment analysis revealed crossover responses among site-years. Line-4 combined the highest overall mean yield with the lowest WAAS and retained the highest mean yield across the five replicated site-years. Rank changes for several other genotypes after excluding the preliminary environments support cautious interpretation of seven-environment stability patterns and continued replicated regional testing.

Background

Bread wheat (*Triticum aestivum* L.) is a major cereal crop whose genetic improvement depends not only on high yield potential but also on the ability of new genotypes to maintain consistent performance across contrasting production environments. Grain yield is quantitatively inherited and strongly influenced by environmental conditions, so genotype × environment ($G \times E$) interaction can alter genotype rankings and complicate selection for broad adaptation. Recent multi-environment wheat

studies have repeatedly shown significant genotype, environment, and $G \times E$ effects, emphasizing the need to evaluate advanced breeding material across representative environments rather than relying on single-environment performance [1–6].

$G \times E$ interaction occurs when genotypes differ in the magnitude or direction of their responses to environmental change. Crossover interactions are especially important for breeding because they may cause the relative ranking of genotypes to change among test environments, thereby creating broad- and specific-adaptation patterns. In Türkiye, multi-environment studies have likewise demonstrated marked environmental and interaction effects in bread wheat. Mut et al. [7] reported both crossover and non-crossover interaction patterns in the Central Black Sea Region, while Karaman et al. [8] showed that AMMI and GGE biplot analyses could discriminate stable and environment-responsive bread wheat genotypes across six Turkish test environments. These findings support the use of complementary stability analyses when selecting advanced lines for regional breeding programs. Related Turkish studies have also documented $G \times E$ effects and yield-stability patterns across environments [9–10].

Mean yield alone does not describe the structure of $G \times E$ interaction, and several complementary statistical approaches are therefore used in multi-environment trials. AMMI partitions the interaction through interaction principal components, whereas GGE biplot analysis focuses on genotype main effects plus $G \times E$ and enables visualization of mean performance, stability, and environment-specific responses [3, 11–14]. Mixed-model procedures provide an additional framework in which random genetic and interaction effects can be estimated by REML and predicted by BLUP. Recent wheat studies have extended this approach through factor-analytic mixed models and BLUP-based GGE analyses, particularly for complex or unbalanced multi-environment datasets [6, 15–16].

Mean performance and stability should be considered jointly when advanced wheat material is evaluated over contrasting environments. The weighted average of absolute scores (WAAS) summarizes the contribution of multiple AMMI interaction principal components into a single stability statistic, with lower values indicating lower interaction across environments [17]. In parallel, mixed-model and BLUP approaches remain useful when replicated plot-level data are available. Recent wheat studies have emphasized the value of combining AMMI, GGE biplots, mixed-model predictions, and interaction-stability measures rather than relying on a single statistic [18–21].

The same set of 25 genotypes was evaluated across three consecutive seasons, but the number of locations increased as the material advanced through the testing pipeline. Bafra and Tokat were represented in 2022–2023 and 2023–2024, while Bafra, Çarşamba, and Tokat were included in 2024–2025. Accordingly, the objectives of this study were to: (i) quantify grain-yield performance of the 25 genotypes across seven location $\times$ year environments; (ii) characterize multi-year $G \times E$ patterns and yield stability using AMMI, WAAS, and GGE biplot approaches; (iii) identify genotypes with broad or environment-specific adaptation across the three seasons; (iv) assess the robustness of mean-performance rankings by excluding the two unreplicated 2022–2023 preliminary environments in a

replicated-environment sensitivity analysis; and (v) retain a detailed plot-level mixed-model assessment of grain yield, hectoliter weight, and thousand-kernel weight for the fully replicated 2024–2025 season.

Methods

Plant material and experimental environments

The study included 20 advanced bread wheat lines (Line-1–Line-20) and five check cultivars: Altındane, Kirve, Nevzatbey, Pandas, and Flamura-85. Thus, a total of 25 genotypes were evaluated. The same set of 25 genotypes was evaluated across the 2022–2023, 2023–2024, and 2024–2025 seasons. In the 2022–2023 preliminary trials, the relevant set consisted of 20 candidate entries (codes 1–20) and five common checks (codes 501–505); the same biological material was subsequently evaluated in the replicated yield trials and is reported here using the Line-1–Line-20 and cultivar designations used in the current manuscript. Pedigree information is presented in Table 1.

Table 1
Pedigree information for the advanced lines and check cultivars used in the study

Genotype	Pedigree / material status
Line-1	DRAGANA/4/BACANORA/3/MASON/JGR//PECOS
Line-2	DRAGANA/4/BACANORA/3/MASON/JGR//PECOS
Line-3	BR12HA/7/3114606//AID/3/CAZO/4/KS9407/6-6-7/4/
Line-4	MIRONIVSKA RANNOSTYGLA/ZAGHOS/3/T 98 - 9//VOM
Altindane	Check cultivar
Line-5	BARAK/IR42//BABAX*2/4/SNI/TRAP/1/3/KAUZ*2/TR4
Line-6	KS14DH0024-28
Line-7	OK14124-2
Line-8	PANDAS/ALTINDANE
Nevzatbey	Check cultivar
Line-9	PANDAS/ALTINDANE
Line-10	PANDAS/ALTINDANE
Line-11	PANDAS/ALTINDANE
Line-12	PANDAS/ALTINDANE
Kirve	Check cultivar
Line-13	PANDAS/ALTINDANE
Line-14	PANDAS/ALTINDANE
Line-15	PANDAS/ALTINDANE
Line-16	PANDAS/ALTINDANE
Flamura-85	Check cultivar
Line-17	PANDAS/ALTINDANE
Line-18	PANDAS/ALTINDANE
Line-19	PANDAS/ALTINDANE
Line-20	PANDAS/ALTINDANE
Pandas	Check cultivar

Note: The designation Line-1–Line-20 was based on the order of the advanced lines after excluding the check cultivars from the pedigree list provided; check cultivars are shown by their cultivar names.

The overall breeding and multi-environment evaluation workflow, including the analytical separation between the seven-site-year stability analyses and the 2024–2025 replicated-data analyses, is summarized in Fig. 1.

The multi-year evaluation comprised seven location × year environments. In 2022–2023, the material was evaluated in Bafra (Samsun) in the preliminary yield trial (ÖVD) and in Tokat in the ÖVD-SA trial. In 2023–2024, the same 25-genotype set was evaluated in replicated yield trials in Bafra and Tokat. In 2024–2025, the experiment was expanded to Bafra, Çarşamba (Samsun), and Tokat. Thus, Bafra and Tokat contributed observations in all three seasons, whereas Çarşamba was included in the final season. The reference coordinates used for the 2024–2025 sites were 41.5678° N, 35.9069° E and 17 m elevation for Bafra; 41.1989° N, 36.7219° E and 15 m for Çarşamba; and 40.3089° N, 36.5529° E and 623 m for Tokat (Kazova). In 2024–2025, sowing was carried out on 10 November 2024 in Bafra, 12 November 2024 in Çarşamba, and 13 November 2024 in Tokat; harvests were conducted on 5 July 2025, 7 July 2025, and 10 July 2025, respectively. The complete three-season trial structure is summarized in Table 2.

Detailed monthly precipitation and mean temperature data were available for the 2024–2025 season and are retained to characterize the final-season environments. Climate data were obtained from the Turkish State Meteorological Service (MGM), 10th Regional Directorate, Samsun, under the Republic of Türkiye Ministry of Environment, Urbanization and Climate Change, and the long-term period was defined as 1950–2025. Cumulative precipitation from November 2024 to July 2025 was 533.8 mm in Bafra, 701.5 mm in Çarşamba, and 295.4 mm in Tokat; the corresponding long-term November–July totals were approximately 519.8, 592.7, and 368.8 mm. Mean temperatures for the November–July trial period/long-term period were 12.8/13.3°C in Bafra, 13.0/12.7°C in Çarşamba, and 11.5/10.4°C in Tokat. Figure 2 therefore represents the 2024–2025 environmental characterization rather than the complete three-season climate record.

Table 2
Multi-year structure of the seven location × year environments included in the study

Season	Location	Trial stage	Replication / record structure	Use in analysis
2022–2023	Bafra	ÖVD	One observed plot per relevant entry	Three-year grain-yield matrix
2022–2023	Tokat	ÖVD-SA	One observed plot per relevant entry	Three-year grain-yield matrix
2023–2024	Bafra	Yield trial	4-replication RCBD; n = 100	Three-year grain-yield matrix
2023–2024	Tokat	Yield trial	4-replication RCBD; n = 100	Three-year grain-yield matrix
2024–2025	Bafra	Yield trial	4-replication RCBD	Three-year yield + final-season mixed model
2024–2025	Çarşamba	Yield trial	4-replication RCBD	Three-year yield + final-season mixed model
2024–2025	Tokat	Yield trial	4-replication RCBD	Three-year yield + final-season mixed model

Environment was defined as a specific location × growing-season combination. The 2022–2023 ÖVD and ÖVD-SA observations represent the preliminary testing stage, whereas the 2023–2024 and 2024–2025 yield trials provided replicated evaluations.

Soil properties were characterized from available site analyses. The Bafra and Çarşamba soils were generally clay loam and slightly alkaline. In Bafra, pH was 7.57 and organic matter was 2.29%; in Çarşamba, pH was 7.86 and organic matter was 1.76%. The Tokat-Kazova site was described as clay loam, with 1.5% organic matter and a pH of 7.9. Because the compiled records did not contain a common sampling year, sampling depth, and analytical protocol for all three sites, these values are used only as historical site descriptors for interpreting location effects and are not treated as season-specific soil measurements.

Experimental design, crop management, and traits evaluated

The experimental structure differed among seasons because the material progressed from preliminary yield testing to replicated multi-location trials. In 2022–2023, the relevant 25-entry set was represented by one observed plot per entry in the Bafra ÖVD and Tokat ÖVD-SA datasets. In 2023–2024, the same material was evaluated in Bafra and Tokat in randomized complete block designs with four replications, giving 100 grain-yield observations per environment (25 genotypes × 4 replications). The Bafra and Tokat grain-yield datasets contained complete records for all 25 genotypes across all four replications, and no grain-yield values were imputed. In 2024–2025, trials were established in Bafra, Çarşamba, and Tokat in

randomized complete block designs with four replications, again giving 100 grain-yield observations per environment. Across seasons, grain yield was expressed as kg ha⁻¹. Because replication structure was not identical across all seven environments, the seven-environment G × E analysis used genotype × environment means rather than imposing an artificial common plot-level replication structure across years. This mean-based approach does not model the different precision of unreplicated and replicated environments; consequently, the seven-environment AMMI, WAAS, and GGE results are interpreted descriptively and are complemented by the replicated-environment sensitivity analysis described below. The detailed 2024–2025 plot-level dataset was retained for the secondary mixed-model analyses of grain yield, hectoliter weight, and thousand-kernel weight. Each replicated-trial plot measured 1.2 × 5.0 m (6 m²). Sowing was carried out at approximately 200–220 kg seed ha⁻¹. A total of 120–140 kg N ha⁻¹ and 60–80 kg P₂O₅ ha⁻¹ was applied; all phosphorus and part of the nitrogen were applied at sowing, and the remaining nitrogen was top-dressed during tillering to stem elongation. Weed, disease, and pest control was carried out as needed in accordance with local agronomic practices and registered product labels.

After harvest, grain yield was determined from the grain harvested from each plot and converted to kg ha⁻¹ according to the harvested plot area. Hectoliter weight and thousand-kernel weight were measured on harvested grain samples in the 2024–2025 breeding trials and expressed as kg hL⁻¹ and g, respectively. The archived trial records did not retain the instrument/model or detailed subsampling protocol for these two physical grain traits; accordingly, they are interpreted as final-season breeding-program measurements rather than as method-standardized comparisons across years. These three traits were retained for the quantitative analyses reported in this study.

Table 3
Actual numbers of observations and replications for traits recorded in the 2024–2025 final-season dataset

Trait	Bafra	Çarşamba	Tokat	Analysis in the manuscript
Grain yield	100 4 rep.	100 4 rep.	100 4 rep.	Combined plot-level mixed model; G × E, BLUP and H ² ; these grain-yield data also contributed to the seven-environment AMMI/WAAS/GGE analysis.
Hectoliter weight	100 4 rep.	100 4 rep.	100 4 rep.	Combined plot-level mixed model; G × E, BLUP, and H ²
Thousand-kernel weight	100 4 rep.	100 4 rep.	100 4 rep.	Combined plot-level mixed model; G × E, BLUP, and H ²

The observation counts in Table 3 apply to the 2024–2025 season. Grain yield, hectoliter weight, and thousand-kernel weight were available for all 25 genotypes in four replications at each of the three environments.

A combined analysis of variance (ANOVA) was additionally performed for the fully replicated 2024–2025 dataset. In the F tests reported in Table 4, the environment effect was tested against replication within

environment, the genotype effect against $G \times E$, and the $G \times E$ effect against the residual mean square. This ANOVA is interpreted only for the three 2024–2025 environments with four replications.

Genotypic repeatability of environment means was calculated as $H^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{G \times E}/E + \sigma^2_e/(Er)]$, where σ^2_G is the genotypic variance, $\sigma^2_{G \times E}$ is the genotype $\times$ environment variance, σ^2_e is the residual variance, $E = 3$ environments, and $r = 4$ replications. H^2 is therefore interpreted here as genotypic repeatability of environment means within the 2024–2025 replicated dataset, not as a three-year broad-sense heritability estimate.

Multi-year and final-season statistical analyses

For the primary seven-environment grain-yield analysis spanning three growing seasons, each location $\times$ year combination was treated as a distinct environment, resulting in a complete matrix of 25 genotypes $\times$ 7 environments. Because the 2022–2023 preliminary trials and the later replicated yield trials differed in experimental precision, the multi-year stability analysis was conducted on genotype $\times$ environment mean grain yields rather than by imposing a single common replication model across all years. Arithmetic environment means, genotype means, and seasonal means were calculated directly from the observed records. The seven-environment matrix was then used for AMMI, WAAS, and GGE analyses. This approach preserves the observed multi-year response pattern while avoiding artificial reconstruction of replication records that were not present in the 2022–2023 trials.

For the fully replicated 2024–2025 subset, plot-level observations for grain yield, hectoliter weight, and thousand-kernel weight were additionally analyzed using a linear mixed-model framework. Genotype was treated as a fixed effect in the primary model, while environment, replication within environment, and $G \times E$ were treated as random effects: $Y_{ijk} = \mu + G_i + E_j + (G \times E)_{ij} + \text{Replication}_k(E_j) + \varepsilon_{ijk}$. Variance components were estimated by REML. A secondary model treating genotype as random was used to obtain BLUPs and genotypic repeatability estimates across the three 2024–2025 environments. These estimates are interpreted specifically for the final-season replicated dataset and are not presented as three-year heritability estimates.

Multi-year stability and adaptation analyses

AMMI analysis was applied to the seven-environment grain-yield mean matrix following the standard AMMI framework [22]. The $G \times E$ interaction matrix was obtained after removing genotype and environment main effects and was decomposed by singular value decomposition. The weighted average of absolute scores (WAAS) was calculated from the absolute genotype scores of all retained interaction principal components, weighted by the percentage of $G \times E$ variation explained by each component [17]; lower WAAS values indicate lower interaction and therefore greater stability. GGE biplot analysis was performed on the environment-centered genotype + $G \times E$ matrix following the GGE framework [23]. The first two GGE principal components were used to visualize genotype responses and relationships among

the seven site-years. Because the 2022–2023 trials were preliminary and unreplicated for the relevant entries, the multi-year AMMI, WAAS, and GGE results are interpreted as comparative stability and adaptation summaries rather than as replicated significance tests for all seven environments. To assess the influence of the two unreplicated preliminary environments on mean-performance ranking, a sensitivity analysis was additionally performed after excluding Bafra 2022–2023 and Tokat 2022–2023. For each genotype, the mean across the five replicated site-years (Bafra and Tokat in 2023–2024; Bafra, Çarşamba, and Tokat in 2024–2025) was calculated with equal weight for each site-year as $[2 \times (2023\text{--}2024 \text{ seasonal mean}) + 3 \times (2024\text{--}2025 \text{ seasonal mean})]/5$. Genotype ranks from the five replicated site-years were compared with ranks from the seven-site-year overall means using Spearman's rank correlation. This sensitivity analysis evaluates the robustness of mean-performance ranking only and is not presented as a replicated sensitivity test of the seven-environment AMMI, WAAS, or GGE stability statistics.

2024–2025 multi-trait analyses

The 2024–2025 grain yield, hectoliter weight, and thousand-kernel weight data were also used for a descriptive multi-trait assessment. Genotype means across the three final-season environments were standardized to z-scores for PCA, and Pearson correlations were calculated among the three traits. These analyses provide complementary information on physical grain traits in the final season but are kept analytically separate from the seven-environment grain-yield matrix. All statistical calculations were performed in Python 3.13.5 using NumPy for matrix operations, SciPy for basic statistical tests, statsmodels for the 2024–2025 mixed-model analyses, and scikit-learn for PCA.

Use of generative AI-assisted technologies

During the preparation of this manuscript, ChatGPT (OpenAI) was used to assist with English-language editing, improvement of readability and clarity, manuscript organization, and formatting. The tool was not used to generate or modify the experimental data, perform the statistical analyses, or make scientific decisions regarding the interpretation of the results. All AI-assisted suggestions were critically reviewed and, where necessary, revised by the author. The author verified the scientific content, statistical results, references, interpretations, and conclusions and takes full responsibility for the final content of the manuscript.

Results

Grain-yield performance across seven site-years

Across the complete 25-genotype $\times$ 7-environment grain-yield matrix, the overall mean was 6,063.5 kg ha⁻¹. Environment means ranged from 2,883.7 kg ha⁻¹ in Bafra 2022–2023 and 4,311.9 kg ha⁻¹ in Tokat 2022–2023 to 6,988.9 kg ha⁻¹ in Bafra 2023–2024, 8,795.3 kg ha⁻¹ in Tokat 2023–2024, 7,162.9 kg ha⁻¹ in Bafra 2024–2025, 5,848.8 kg ha⁻¹ in Çarşamba 2024–2025, and 6,453.1 kg ha⁻¹ in Tokat 2024–2025.

The wide range among site-year means shows that the material was exposed to markedly contrasting production environments over the three seasons. Line-4 had the highest overall mean yield across seven site-years at 6,794.3 kg ha⁻¹, followed by Line-9 at 6,630.3 kg ha⁻¹, Kirve at 6,545.6 kg ha⁻¹, Line-16 at 6,452.2 kg ha⁻¹, and Line-18 at 6,432.0 kg ha⁻¹.

Detailed 2024–2025 plot-level mixed-model results

Table 4
Combined analysis of variance for the replicated 2024–2025 final-season dataset

Source of variation	df	Grain yield MS (P)	Hectoliter weight MS (P)	Thousand-kernel weight MS (P)
Environment	2	43268603 (0.0153)	115.61 (0.0010)	1445.34 (< 0.0001)
Replication (Environment)	9	6274416 (< 0.0001)	7.04 (< 0.0001)	4.87 (0.3584)
Genotype	24	2709543 (0.0453)	26.84 (< 0.0001)	127.87 (< 0.0001)
G × E	48	1526477 (0.0001)	4.94 (< 0.0001)	16.18 (< 0.0001)
Error	216	715826	1.71	4.39

df: degrees of freedom; MS: mean square; values in parentheses are p-values. This table refers specifically to the 2024–2025 four-replication dataset. The environment effect was tested against Replication(E), the genotype effect against G × E, and the G × E effect against the residual mean square.

Within the replicated 2024–2025 subset, REML-estimated variance components for grain yield were 98,589 for genotypic variance, 202,663 for G × E variance, and 715,826 for residual variance, resulting in H² = 0.437 across the three final-season environments. H² was 0.816 for hectoliter weight and 0.873 for thousand-kernel weight. These values therefore describe repeatability within the 2024–2025 Bafra, Çarşamba, and Tokat trials rather than across all three years. For grain-yield BLUPs in that final season, Line-4, Line-1, and Line-6 remained among the highest predicted genotypes, whereas Line-9 had the highest BLUP for both hectoliter weight and thousand-kernel weight (Table 5).

Table 5
BLUP estimates for grain yield, hectoliter weight, and thousand-kernel weight
in the replicated 2024–2025 dataset

Genotype	Grain yield BLUP (kg ha⁻¹)	Hectoliter weight BLUP (kg hL⁻¹)	TKW BLUP (g)
Line-1	6821.2	77.17	45.46
Line-2	6729.6	75.74	44.33
Line-3	6535.8	75.84	46.98
Line-4	6852.1	78.39	45.65
Line-5	6322.7	76.01	41.56
Line-6	6786.9	75.40	44.15
Line-7	6679.6	76.15	34.27
Line-8	6574.3	77.50	45.67
Line-9	6494.6	79.41	48.09
Line-10	6493.4	77.98	42.80
Line-11	6320.0	78.32	43.42
Line-12	6365.1	78.05	44.97
Line-13	6194.1	78.69	44.30
Line-14	6626.5	76.35	43.24
Line-15	6485.2	79.07	45.53
Line-16	6381.5	77.03	41.66
Line-17	6468.2	78.66	47.47
Line-18	6498.8	78.69	47.31
Line-19	6363.9	76.82	44.00
Line-20	6140.5	76.42	44.08
Altındane	6169.5	76.28	43.66
Kirve	6749.0	76.38	40.56
Nevzatbey	6594.1	75.66	41.31
Pandas	6323.0	76.62	41.71
Flamura-85	6236.9	76.07	45.60

REML variance components and genotypic repeatability (H^2) shown here apply only to the three replicated 2024–2025 environments ($nE = 3$, $r = 4$). They are retained as a detailed final-season analysis and should not be interpreted as three-year heritability estimates.

In the 2024–2025 replicated subset, the overall mean grain yield was 6,488.3 kg ha⁻¹. Environment means were 7,162.9 kg ha⁻¹ in Bafra, 6,453.1 kg ha⁻¹ in Tokat, and 5,848.8 kg ha⁻¹ in Çarşamba. The highest final-season overall grain yield was recorded for Line-4 at 7,321.5 kg ha⁻¹; Line-1, Line-6, Kirve, and Line-2 were also among the higher-yielding genotypes in that season.

When all three seasons were considered together, genotype rankings changed because the early preliminary environments and the later replicated environments differed substantially in productivity. Seasonal means and seven-environment overall means are summarized in Table 6. Line-4 retained the highest overall mean across the seven site-years, whereas Line-9 and Kirve also maintained high multi-year means. The seven-site-year dataset therefore provides a broader descriptive view of multi-year response, while the replicated-environment sensitivity analysis below evaluates how strongly the two preliminary environments influenced mean-performance ranking.

Table 6

Seasonal mean grain yields, seven-site-year overall means, and replicated-environment sensitivity means for the 25 genotypes

Genotype	2022– 2023 mean	2023– 2024 mean	2024– 2025 mean	Overall mean across 7 site- years	Mean across 5 replicated site-years (rank)
Line-1	2974.9	8516.7	7250.7	6390.7	7757.1 (2)
Line-2	2506.0	8687.5	7041.0	6215.7	7699.6 (3)
Line-3	4244.2	8255.6	6597.2	6398.7	7260.6 (10)
Line-4	4218.7	8579.2	7321.5	6794.3	7824.6 (1)
Line-5	3816.1	9512.5	6109.0	6426.3	7470.4 (7)
Line-6	1098.5	8397.2	7172.2	5786.9	7662.2 (4)
Line-7	2753.0	8437.5	6926.4	6165.7	7530.8 (5)
Line-8	3003.4	7416.7	6685.4	5842.3	6977.9 (16)
Line-9	5639.4	7812.5	6502.8	6630.3	7026.7 (14)
Line-10	3232.4	7404.2	6500.0	5824.8	6861.7 (18)
Line-11	3654.3	6566.7	6102.8	5535.8	6288.4 (23)
Line-12	4657.3	8233.3	6206.2	6342.8	7017.0 (15)
Line-13	2824.7	8070.8	5814.6	5605.0	6717.1 (20)
Line-14	4023.7	7757.6	6804.9	6282.5	7186.0 (12)
Line-15	4562.6	7620.8	6481.3	6258.7	6937.1 (17)
Line-16	4222.6	8994.4	6243.7	6452.2	7344.0 (9)
Line-17	3924.4	8200.0	6442.3	6225.1	7145.4 (13)
Line-18	3697.5	9045.8	6512.5	6432.0	7525.8 (6)
Line-19	3320.4	8731.2	6203.5	6102.0	7214.6 (11)
Line-20	2006.8	8466.7	5691.7	5431.7	6801.7 (19)
Altındane	4392.4	7116.7	5758.4	5756.2	6301.7 (22)
Kirve	4273.1	8008.3	7085.4	6545.6	7454.6 (8)
Nevzatbey	2436.2	5829.2	6730.6	5246.1	6370.0 (21)
Pandas	4565.3	5462.5	6109.7	5483.6	5850.8 (25)
Flamura-85	3896.7	6179.2	5912.5	5412.8	6019.2 (24)

Yield values are given as kg ha⁻¹. The 2022–2023 mean is based on Bafra ÖVD and Tokat ÖVD-SA; the 2023–2024 mean is based on Bafra and Tokat; the 2024–2025 mean is based on Bafra, Çarşamba, and Tokat. The overall mean gives equal weight to all seven location × year environments and is not an equal-weight average of the three seasonal means. The five-replicated-site-year mean excludes the two 2022–2023 preliminary environments and gives equal weight to the two 2023–2024 and three 2024–2025 site-years; the number in parentheses is the genotype rank within this replicated-environment sensitivity analysis.

Sensitivity analysis restricted to the five replicated site-years

Excluding the two unreplicated 2022–2023 preliminary environments increased the overall mean grain yield across the remaining five replicated site-years to 7,049.8 kg ha⁻¹. Line-4 remained the highest-yielding genotype (7824.6 kg ha⁻¹), followed by Line-1 (7757.1), Line-2 (7699.6), Line-6 (7662.2), and Line-7 (7530.8 kg ha⁻¹). Genotype rankings based on the seven-site-year and five-replicated-site-year means were positively and significantly associated (Spearman $\rho = 0.705$, $p < 0.001$), indicating overall concordance but also meaningful rank changes after removal of the preliminary environments. Line-4 remained first in both analyses, whereas Line-9 shifted from rank 2 to rank 14, Kirve from rank 3 to rank 8, and Line-16 from rank 4 to rank 9. Thus, the leading mean performance of Line-4 was robust to exclusion of the unreplicated preliminary trials, while the relative ranking of several other genotypes was sensitive to their inclusion. This analysis addresses robustness of mean-performance ranking; the seven-environment AMMI, WAAS, and GGE results remain descriptive summaries of the full multi-year response pattern.

Stability and adaptation across seven site-years

AMMI analysis of the seven-environment grain-yield matrix showed that IPCA1 explained 46.68% of the $G \times E$ interaction sum of squares and IPCA2 explained 20.92%; together, the first two interaction axes represented 67.60% of the $G \times E$ pattern. Because seven site-years were included, additional interaction dimensions remained beyond the first two axes. The AMMI1 genotype plot (Fig. 3) showed that Line-4 combined the highest mean yield with an IPCA1 score very close to zero, whereas several other genotypes displayed larger positive or negative interaction scores.

WAAS values confirmed that high mean yield and low interaction should be considered jointly (Table 7). Line-4 had the lowest WAAS value (110.17) and also ranked first for mean grain yield across the seven site-years. Kirve ranked second for WAAS (338.61) and third for mean yield, while Line-16 ranked fourth for WAAS (463.08) and fourth for mean yield. Line-17 and Line-19 also had relatively low WAAS values, although their mean yields were lower than those of the leading high-yielding genotypes. In contrast, Line-9 had the second-highest overall mean yield but a higher WAAS value (1,140.38), indicating stronger environment-specific response.

Table 7
WAAS stability values and seven-environment grain-yield ranking across seven site-years

Genotype	Mean yield (kg ha⁻¹)	WAAS	WAAS rank	Yield rank
Line-4	6794.3	110.17	1	1
Kirve	6545.6	338.61	2	3
Line-17	6225.1	405.48	3	12
Line-16	6452.2	463.08	4	4
Line-19	6102.0	547.63	5	15
Line-5	6426.3	691.30	6	6
Line-11	5535.8	694.14	7	21
Line-3	6398.7	730.33	8	7
Line-12	6342.8	806.69	9	9
Line-15	6258.7	851.18	10	11
Line-14	6282.5	852.57	11	10
Line-8	5842.3	881.18	12	16
Line-1	6390.7	893.52	13	8
Line-13	5605.0	1009.36	14	20
Line-18	6432.0	1031.72	15	5
Line-9	6630.3	1140.38	16	2
Flamura-85	5412.8	1188.53	17	24
Line-2	6215.7	1402.54	18	13
Line-10	5824.8	1413.67	19	17
Nevzatbey	5246.1	1503.22	20	25
Altındane	5756.2	1541.95	21	19
Line-7	6165.7	1686.75	22	14
Line-6	5786.9	1853.73	23	18
Line-20	5431.7	2078.29	24	23
Pandas	5483.6	2136.30	25	22

WAAS: weighted average of the absolute AMMI interaction scores, weighted by the percentage of $G \times E$ variation explained by each interaction component. Lower WAAS indicates lower interaction. Mean yield and WAAS should be interpreted jointly when selecting broadly adapted material.

In the GGE analysis, PC1 explained 39.82% and PC2 explained 22.65% of the $G + G \times E$ variation; together they represented 62.47% of the multi-year GGE structure (Fig. 4). Clear crossover behavior was observed, with different genotypes attaining the highest numerical yield in different site-years. The highest numerical yields were recorded for Line-9 in Bafra 2022–2023, Altındane in Tokat 2022–2023, Line-5 in Bafra 2023–2024, Line-18 in Tokat 2023–2024, Kirve in Bafra 2024–2025, Line-18 in Çarşamba 2024–2025, and Line-8 in Tokat 2024–2025. These changing environment-specific leaders illustrate the importance of evaluating the same material over both locations and years.

The joint plot of mean grain yield and WAAS (Fig. 5) further distinguished broadly useful genotypes from genotypes whose high performance was accompanied by stronger interaction. Line-4 occupied the most favorable high-yield/low-WAAS position. Kirve and Line-16 also combined above-average yield with comparatively low WAAS. Line-9 had a high overall mean but greater interaction, suggesting that its performance depended more strongly on environment. These multi-year patterns provide a broader basis for selecting material for continued regional testing than the final-season results alone.

2024–2025 physical grain traits and multi-trait relationships

Physical grain traits were available as a complete replicated dataset only for 2024–2025 and are therefore interpreted as final-season complementary information. In that season, the overall mean hectoliter weight was 77.15 kg hL⁻¹. Line-9 (79.92), Line-15 (79.50), Line-13 and Line-18 (both 79.04), and Line-17 (79.00 kg hL⁻¹) showed high values. The significant 2024–2025 $G \times E$ interaction for hectoliter weight indicates that physical grain quality also varied among the three final-season environments.

For 2024–2025, the overall mean thousand-kernel weight was 43.91 g. Çarşamba had the highest environmental mean at 46.87 g, Bafra was intermediate at 45.24 g, and Tokat had the lowest at 39.62 g. Among genotypes, Line-9 (48.70 g), Line-17 (47.98 g), Line-18 (47.80 g), and Line-3 (47.43 g) were notable for high thousand-kernel weight.

Within the 2024–2025 genotype means, grain yield showed weak and nonsignificant relationships with hectoliter weight ($r = -0.120$; $p = 0.569$) and thousand-kernel weight ($r = -0.075$; $p = 0.721$), whereas hectoliter weight and thousand-kernel weight were positively correlated ($r = 0.479$; $p = 0.0154$). These associations describe the final-season dataset and are not intended as three-year trait correlations.

In the PCA of the 2024–2025 standardized genotype means for grain yield, hectoliter weight, and thousand-kernel weight, PC1 explained 50.53% and PC2 32.18% of the total variance (82.71% together; Fig. 6). PC1 was strongly associated with the two physical grain traits, whereas PC2 was determined primarily by grain yield. Thus, the final-season PCA provides a complementary view of simultaneous yield and grain-trait selection but remains separate from the seven-environment yield-stability analysis.

Discussion

Extending the study from a single growing season to three consecutive seasons substantially broadened the environmental range over which the 25 bread wheat genotypes were assessed. The seven site-years included two preliminary environments in 2022–2023, two replicated environments in 2023–2024, and three replicated environments in 2024–2025. Environment means ranged from 2,883.7 to 8,795.3 kg ha⁻¹, demonstrating that year-to-year as well as location-to-location variation strongly affected realized yield. This broader pattern is consistent with multi-environment wheat studies showing that environmental heterogeneity and $G \times E$ interaction can alter genotype rankings and must be considered in regional breeding decisions [2–3, 5, 14].

The three-season dataset is intentionally analyzed differently from the fully replicated 2024–2025 subset because the 2022–2023 ÖVD and ÖVD-SA records represent a preliminary testing stage with one observed plot per relevant entry. Combining all years under a single balanced four-replication model would therefore create a false replication structure. Using genotype $\times$ environment means for the seven-site-year AMMI, WAAS, and GGE analyses preserves the observed response pattern, while the 2024–2025 mixed-model analysis retains the stronger plot-level inference available for the final season. Modern MET frameworks can accommodate imbalance when complete observation-level information and suitable variance structures are available [6, 15–16], but missing historical replication records should not be reconstructed.

The replicated-environment sensitivity analysis directly examined the extent to which the two unreplicated 2022–2023 preliminary trials influenced genotype ordering. After those environments were excluded, Line-4 retained the highest mean yield, and the five-replicated-site-year and seven-site-year rankings remained significantly correlated (Spearman $\rho = 0.705$, $p < 0.001$). However, several genotypes changed position substantially: Line-9 moved from second to fourteenth, Kirve from third to eighth, and Line-16 from fourth to ninth. These changes show that the preliminary environments contributed meaningful information about response under lower-yielding conditions but also affected overall rank ordering because they did not have the same experimental precision as the later trials. Accordingly, the leading mean-performance signal for Line-4 is comparatively robust, whereas seven-environment rankings and stability summaries for other genotypes should be interpreted as descriptive multi-year evidence pending additional replicated site-years.

The environmental progression also helps explain why the multi-year genotype ranking differs from the 2024–2025 final-season ranking. Yields were markedly lower in the 2022–2023 preliminary environments and substantially higher in Tokat 2023–2024, while the three 2024–2025 environments occupied an intermediate range. Because detailed climate records were available in the current dataset only for 2024–2025, the differences among earlier site-years should be interpreted as integrated environment effects rather than being attributed to a single climatic factor. The results nevertheless show that selection based on one season would have provided an incomplete representation of adaptation.

AMMI analysis across seven site-years revealed a complex interaction structure. IPCA1 explained 46.68% and IPCA2 20.92% of the $G \times E$ interaction, so the first two axes together accounted for 67.60%, with additional interaction dimensions remaining. Line-4 was especially notable because it combined the highest overall mean yield across seven site-years with an IPCA1 score near zero and the lowest WAAS value. Kirve and Line-16 also combined relatively high mean yield with low WAAS. In contrast, Line-9 had the second-highest overall mean but a higher WAAS value, indicating stronger environmental responsiveness. This distinction illustrates why mean performance and stability should be evaluated jointly [17–21].

The GGE biplot provided a complementary description of the multi-year response pattern. The first two components explained 62.47% of $G + G \times E$ variation, and the environment vectors separated the early preliminary trials from the later higher-yielding trials. Different genotypes produced the highest numerical yield in different site-years, confirming crossover behavior. Line-9 led in Bafra 2022–2023, Altındane in Tokat 2022–2023, Line-5 in Bafra 2023–2024, Line-18 in Tokat 2023–2024 and Çarşamba 2024–2025, Kirve in Bafra 2024–2025, and Line-8 in Tokat 2024–2025. Similar crossover patterns have been reported in bread wheat MET studies and are central to distinguishing broad from specific adaptation [3, 8, 24].

The joint interpretation of mean yield and WAAS is consistent with the broader multi-environment-trial principle that stability should not be evaluated independently of productivity. The pattern observed for Line-4—high overall yield across seven site-years together with a low interaction score—illustrates the type of combined response targeted by AMMI/WAAS-based selection approaches. Comparable wheat studies using AMMI, BLUP, GGE, and related stability statistics have likewise emphasized simultaneous consideration of mean performance and interaction stability rather than selection on either criterion alone [17, 20–21].

The changing numerical leaders among site-years and the separation of environments in the GGE biplot are characteristic of crossover $G \times E$ interaction. Similar environment-dependent rank changes have been reported in Turkish bread wheat trials [7–8] and in other multi-environment wheat studies [3, 24]. The GGE framework was specifically developed to visualize genotype main effects together with $G \times E$ and to identify environment-specific winning genotypes [23], which supports the interpretation of the site-year differences observed here.

The 2024–2025 physical grain-trait results should be viewed as complementary rather than as a complete three-year quality assessment. Hectoliter weight and thousand-kernel weight were measured with full replication in the final season, and Line-9 showed the highest overall values for both traits. Their high repeatability within 2024–2025 indicates consistent genotypic differentiation across Bafra, Çarşamba, and Tokat during that season, but corresponding data were not available in a common structure for the earlier seasons. Accordingly, the principal multi-year conclusions of this study are based on grain yield, while the physical-trait analysis informs final-season multi-trait selection [25].

Overall, the seven-environment analysis broadens the environmental range used to describe advanced bread wheat responses, while the replicated-environment sensitivity analysis provides an explicit check on the influence of the two unreplicated 2022–2023 preliminary trials. Line-4 ranked first for seven-site-year mean yield, first for WAAS in the full seven-environment analysis, and first for mean yield across the five replicated site-years. In contrast, the rank shifts observed for Line-9, Kirve, Line-16, and several other genotypes show that inclusion of the preliminary environments materially affected genotype ordering. The main limitation therefore remains the heterogeneous experimental precision across years; seven-environment AMMI, WAAS, and GGE patterns should be treated as descriptive evidence of multi-year response, while future release or recommendation decisions should continue to rely on replicated multi-year regional testing.

Conclusions

Evaluation of the same 25 bread wheat genotypes across the 2022–2023, 2023–2024, and 2024–2025 growing seasons expanded the study to seven location × year environments: Bafra and Tokat in the first two seasons and Bafra, Çarşamba, and Tokat in the final season. Across the seven site-years, the overall mean grain yield was 6,063.5 kg ha⁻¹. Line-4 had the highest overall mean yield across the seven site-years (6,794.3 kg ha⁻¹) and the lowest WAAS (110.17). Importantly, Line-4 also retained the highest mean yield in the sensitivity analysis restricted to the five replicated site-years (7,824.6 kg ha⁻¹). The significant rank correlation between the seven-site-year and five-replicated-site-year means (Spearman $\rho = 0.705$, $p < 0.001$) indicates overall agreement, but substantial rank changes for several genotypes show that the two preliminary environments influenced genotype ordering. Therefore, the seven-environment AMMI, WAAS, and GGE results are best interpreted as comparative multi-year stability and adaptation summaries rather than as fully replicated inference across all site-years. Continued replicated multi-year regional testing remains appropriate before final cultivar recommendation or release decisions.

Abbreviations

AMMI	
additive main effects and multiplicative interaction	
BLUP	
best linear unbiased prediction	
GGE	
genotype plus genotype-by-environment interaction	
HLW	
hectoliter weight	
IPCA	
interaction principal component axis	
PCA	
principal component analysis	
REML	

restricted maximum likelihood
TKW
thousand-kernel weight
WAAS
weighted average of absolute scores
ÖVD
preliminary yield trial
ÖVD-SA
semi-arid preliminary yield trial.

Declarations

Ethics approval and consent to participate

This study did not involve human participants or animals. Experimental research and field studies on bread wheat (*Triticum aestivum* L.) were conducted in accordance with relevant institutional, national, and international guidelines and legislation. The plant materials consisted of advanced breeding lines and check cultivars used within the Black Sea Agricultural Research Institute breeding program. No wild or endangered plant species were used.

Consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

Funding

This work was supported by the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM/TA/00/01/04/015, within the project “Bread Wheat Breeding Research for the Black Sea Region”. The funder had no role in the design of the study; collection, analysis, or interpretation of data; preparation of the manuscript; or the decision to submit the work for publication.

Author Contribution

Y.B. conceived and designed the study, curated the data, performed the formal analyses, developed the methodology, prepared the visualizations and figures, interpreted the results, and wrote and revised the manuscript. The author read and approved the final manuscript.

Acknowledgements

Not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the author used ChatGPT (OpenAI) to assist with English language editing, improving readability and clarity, manuscript structuring, and formatting according to the journal guidelines. The scientific content, data, statistical analyses, interpretation of the results, and conclusions were reviewed and verified by the author. The author takes full responsibility for the content of the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

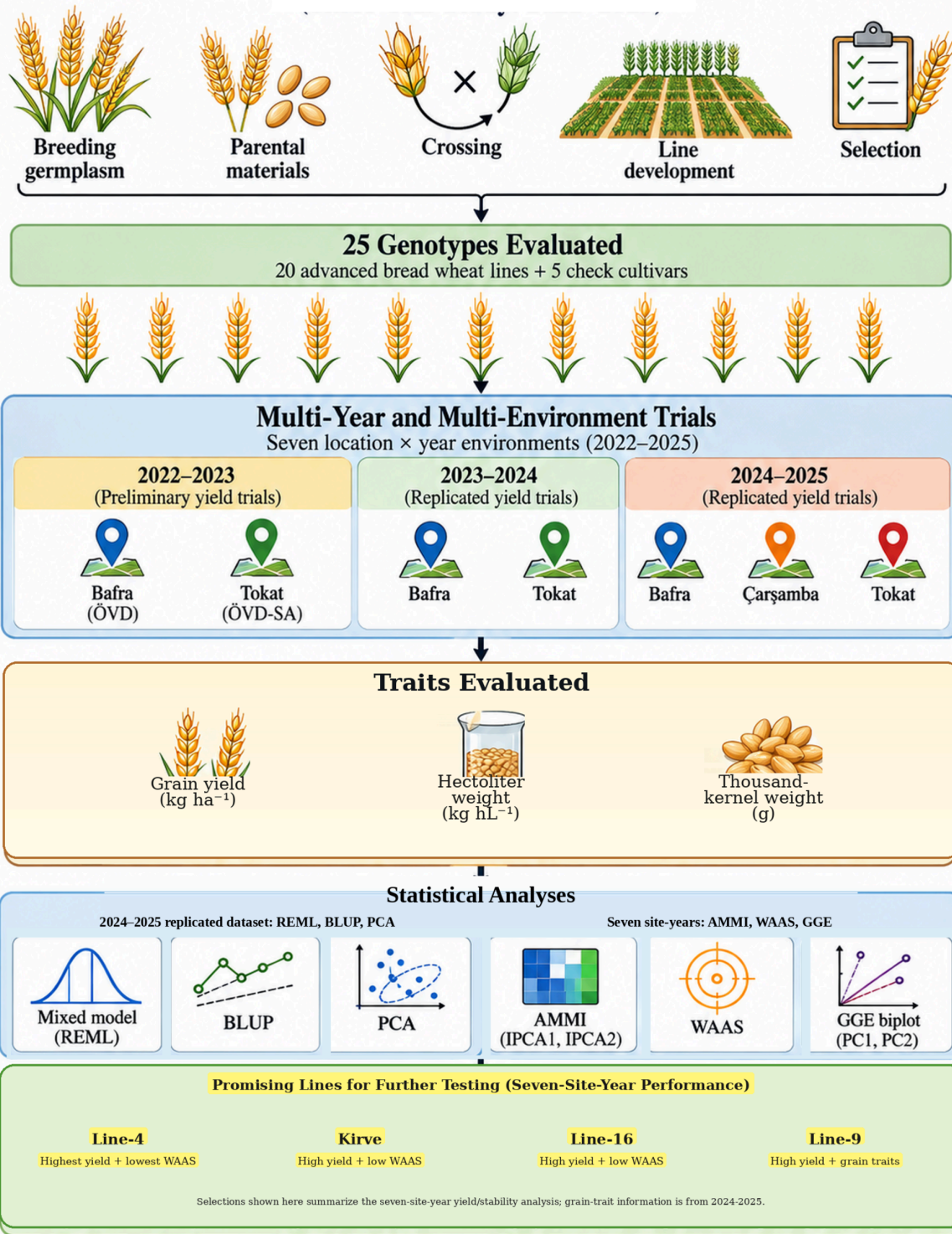


Figure 1

Breeding and multi-environment evaluation workflow for advanced bread wheat lines. AMMI, WAAS, and GGE summarize the seven site-years; REML, BLUP, and PCA refer to the replicated 2024-2025 dataset.

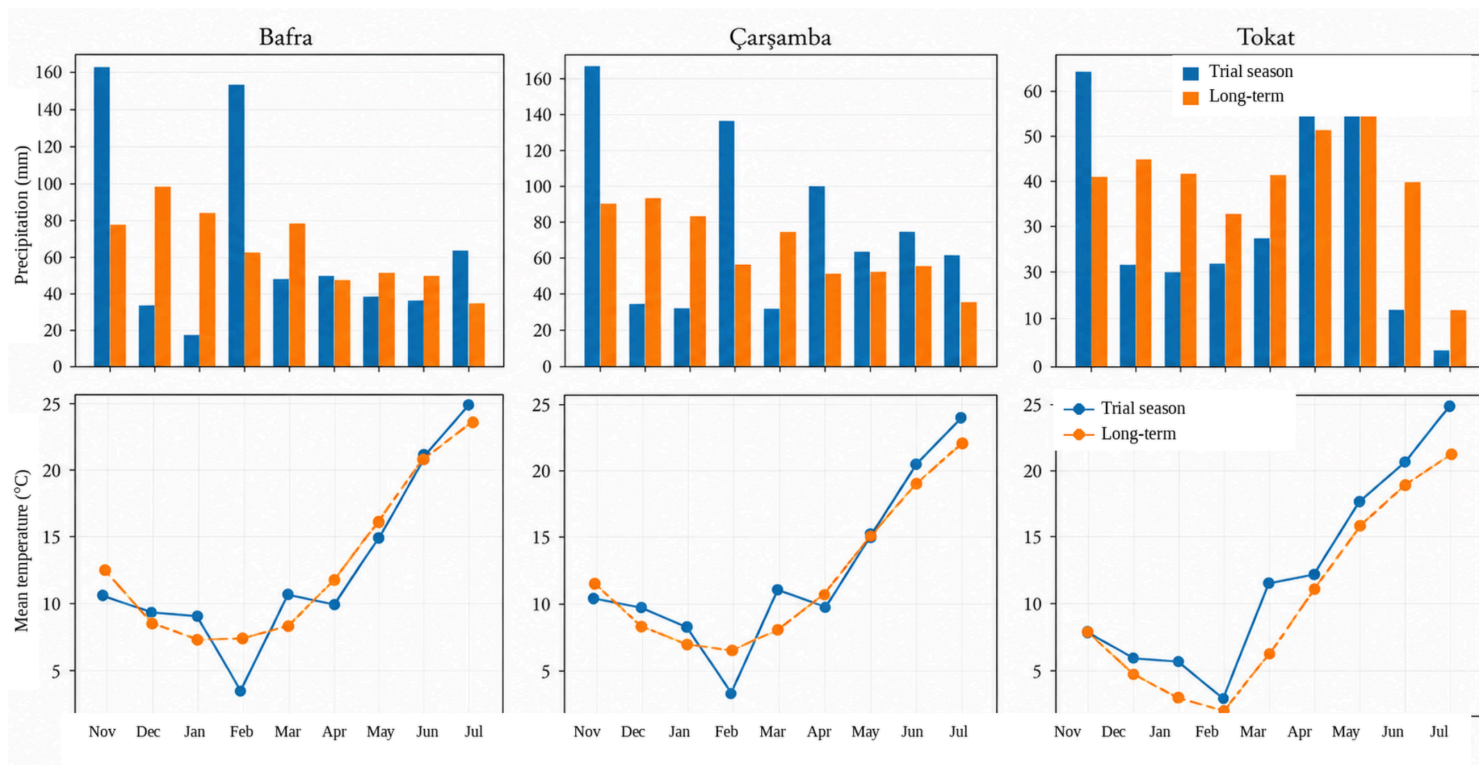


Figure 2

Climate profiles and long-term means across three environments. Monthly precipitation and mean temperature from November to July are shown for Bafra, Çarşamba, and Tokat during 2024-2025 and compared with the 1950-2025 long-term means. This figure characterizes the final season only.

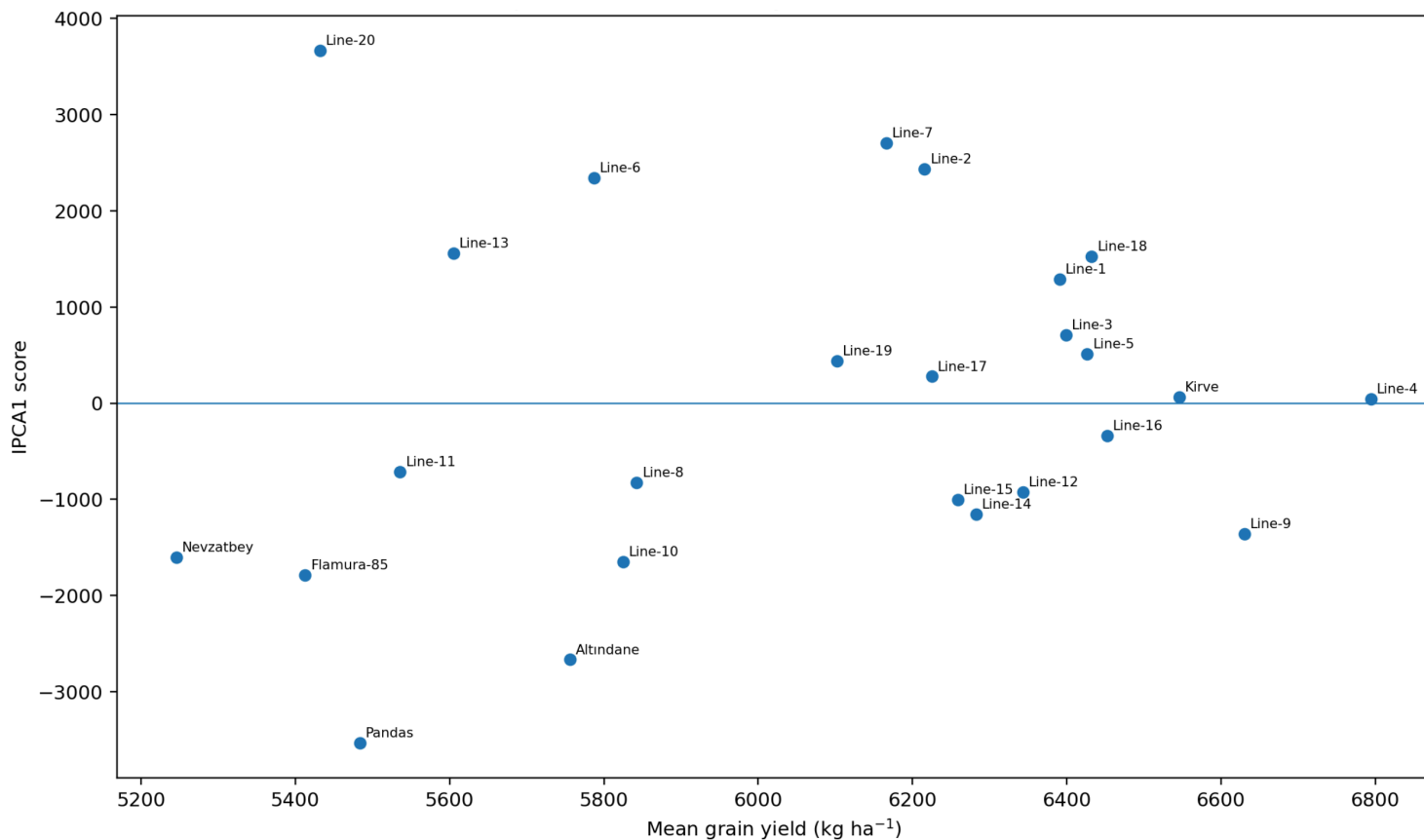


Figure 3

AMMI1 genotype plot of grain yield across seven site-years. Mean grain yield (kg ha⁻¹) is plotted against genotype IPCA1 scores; IPCA1 explains 46.68% of the G × E interaction.

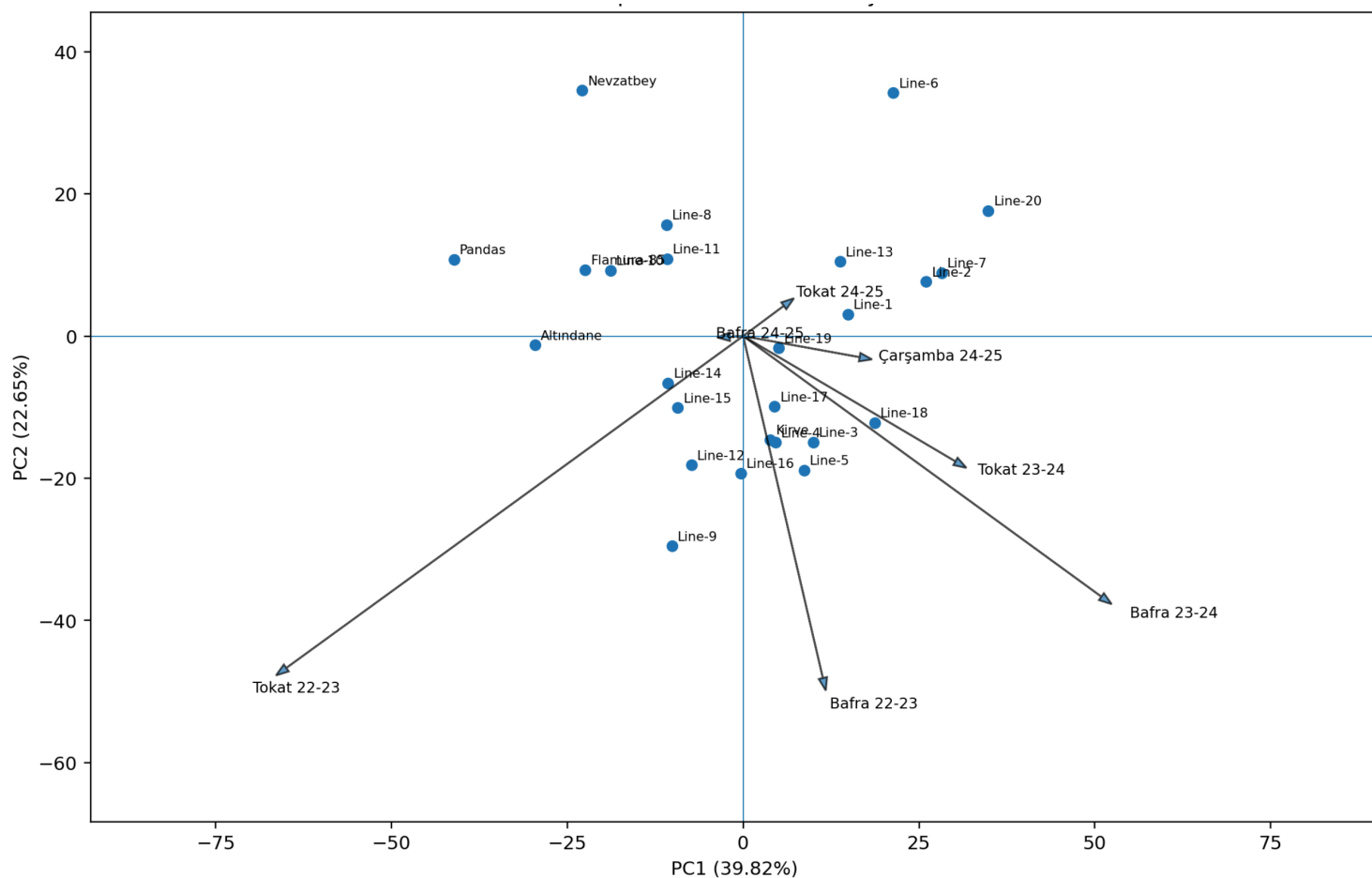


Figure 4

GGE biplot of 25 genotypes across seven site-years. PC1 and PC2 explain 39.82% and 22.65% of the $G + G \times E$ variation, respectively.

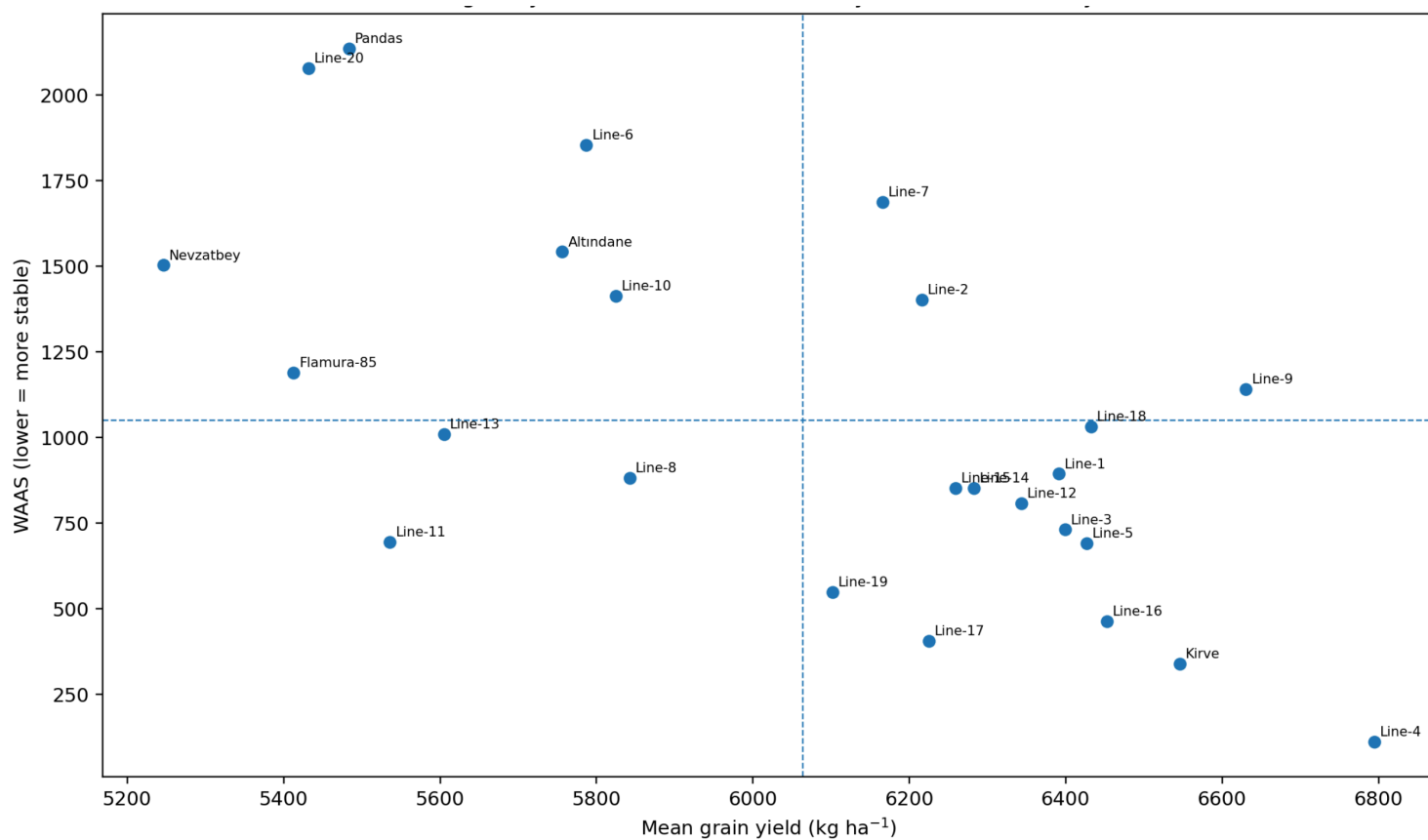


Figure 5

Mean grain yield and WAAS across seven site-years. Lower WAAS values indicate lower genotype × environment interaction; genotypes combining high mean yield and low WAAS are located toward the lower-right portion of the plot.

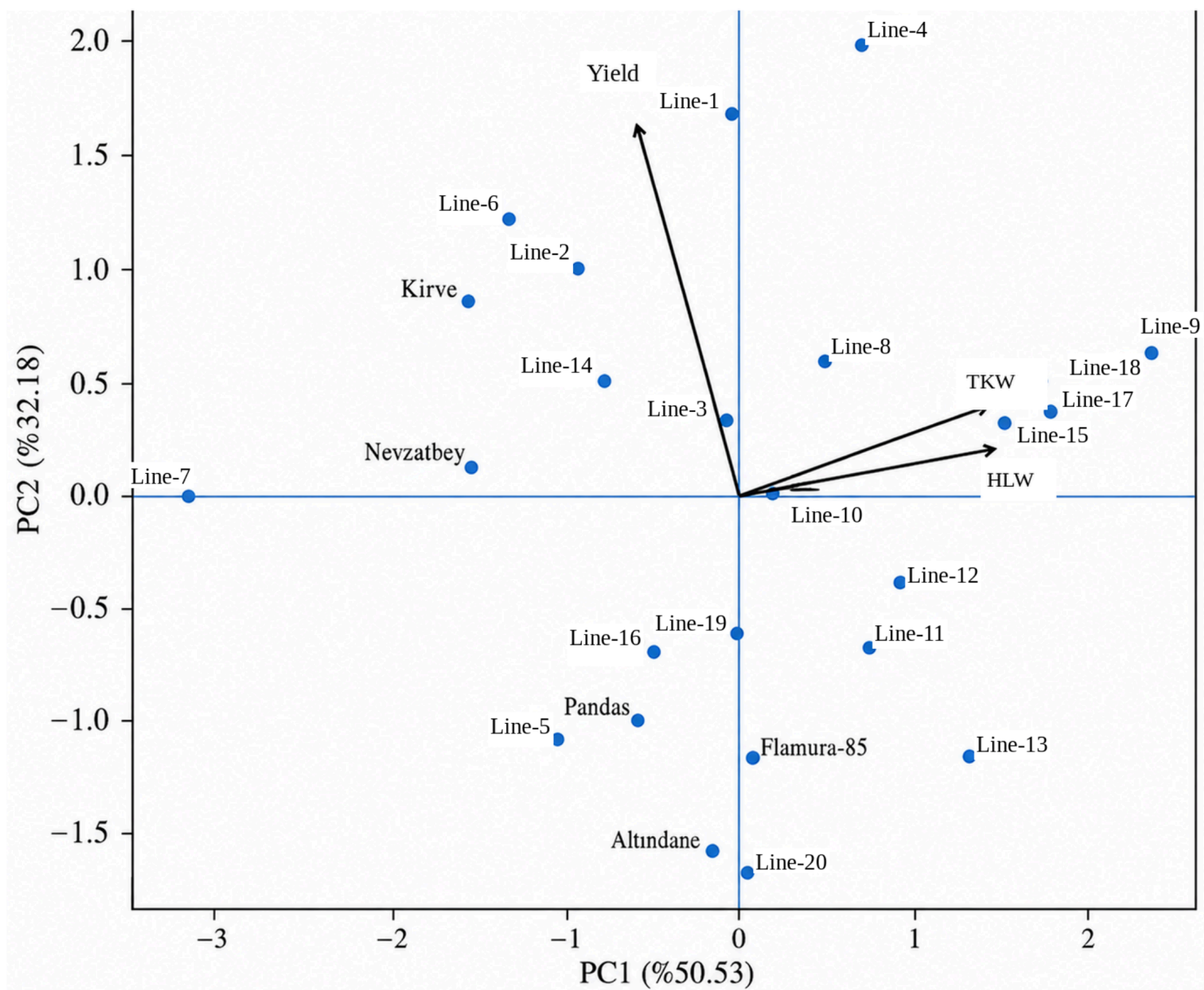


Figure 6

PCA biplot of yield and physical grain traits. Standardized genotype means are shown for grain yield, hectoliter weight (HLW), and thousand-kernel weight (TKW). PC1 explains 50.53% of the total variance, PC2 explains 32.18%, and the first two components together explain 82.71%.